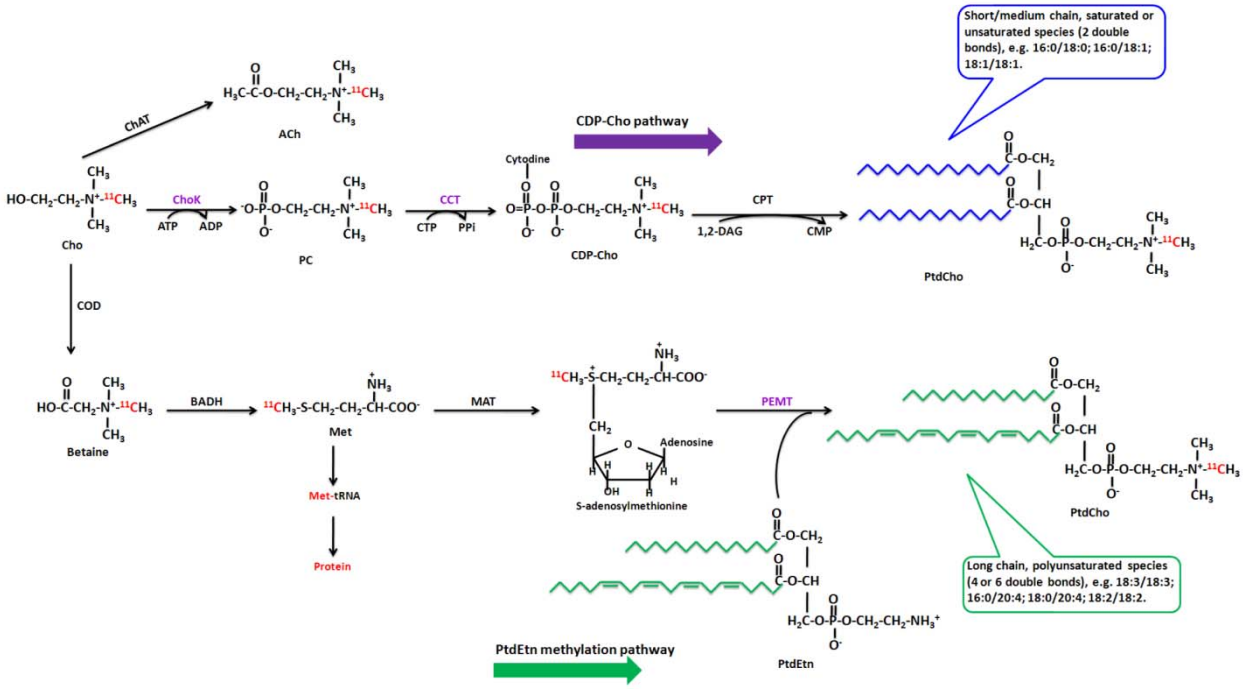
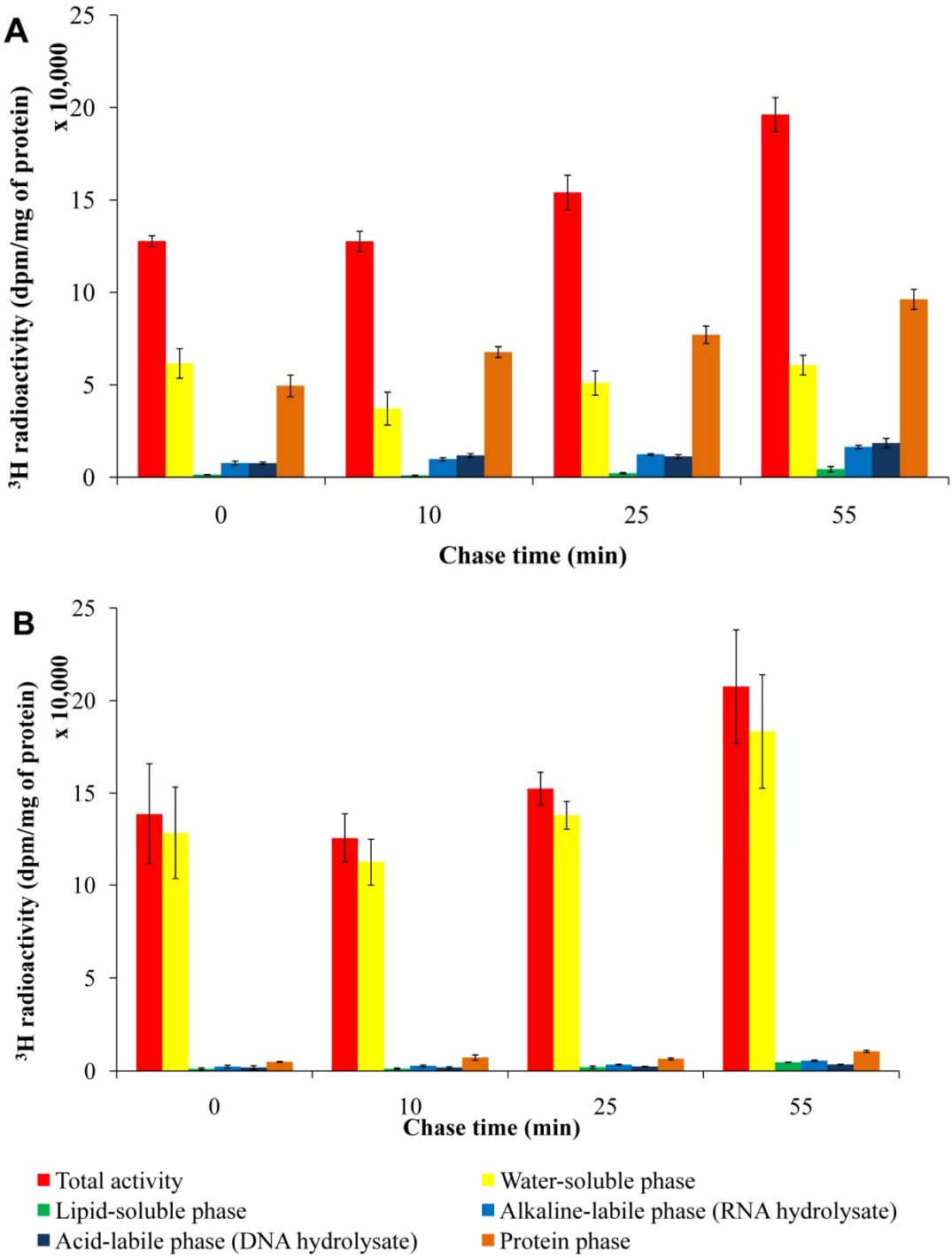


SUPPLEMENTAL FIGURE 1. Metabolic fate of [methyl-^{11}C]-choline. Abbreviation: 1,2-DAG: 1,2-diaclglycerol; Ach: acetylcholine; BADH: betaine aldehyde dehydrogenase; CCT: CTP:phosphocholine cytidylyltransferase; ChAT: choline acetyltransferase; Cho: choline; COD: choline oxidase; CPT: Cholinephosphotransferase; MAT: methionine adenosyltransferase; Met: methionine; PC: phosphocholine; PEMT: phosphatidylethanolamine methyltransferase; PtdCho: phosphatidylcholine; PtdEtn: phosphatidylethanolamine; SAM: s-adenosylmethionine.



SUPPLEMENTAL FIGURE 2. Pulse and chase study in WCH17 cells using L-[methyl-^{3}H]Methionine. (A) Without protein synthesis inhibitor Cycloheximide. (B) With protein synthesis inhibitor Cycloheximide. For Chase time 0 there was only pulse WCH17 cells 5 min and no chase.

SUPPLEMENTAL FIGURE 2

[L-methyl-^{3}H]-Met was used to investigate the contribution of PtdEtn methylation pathway to PtdCho synthesis in HCC. A well-differentiated woodchuck HCC cell line WCH17 was used for this experiment, which was purchased from American Type Culture Collection (ATCC) (Manassas, VA).

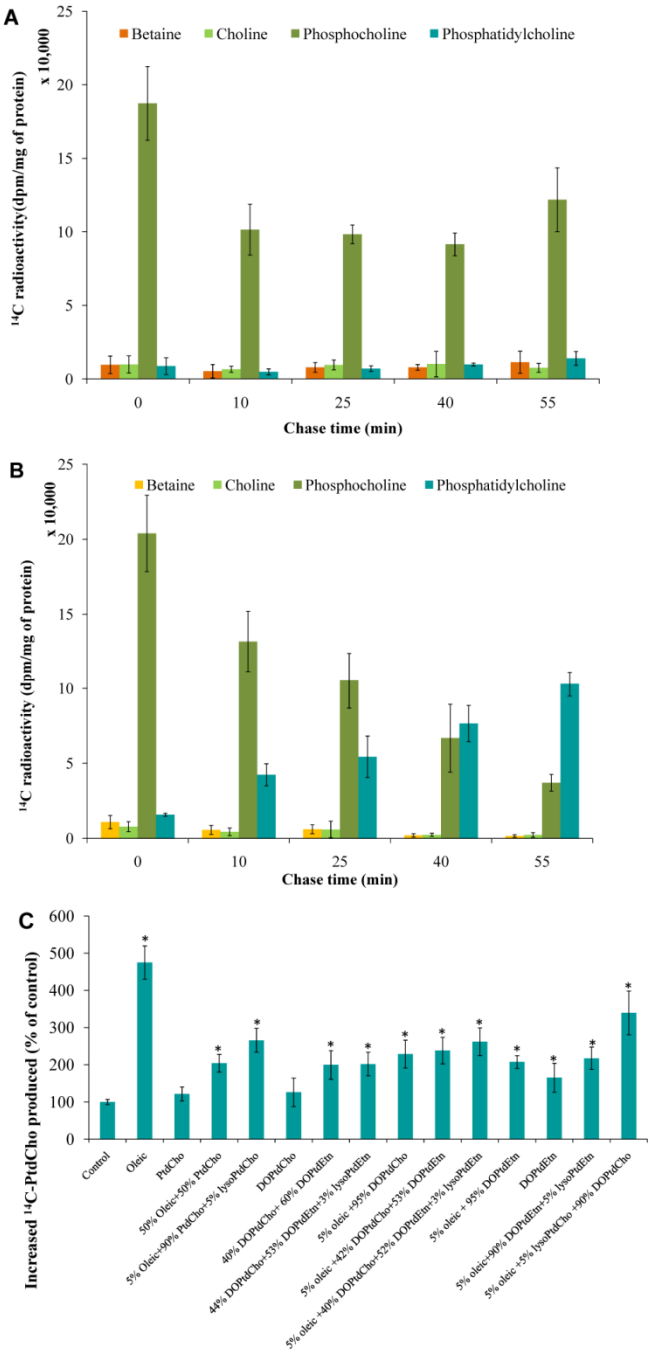
Materials and Methods

1×10^{7} WCH17 cells in 75 cm^{2} corning cell culture flasks were incubated with 10 ml of 370 KBq [methyl-^{3}H]-Met in HBSS at 37°C for 5 min (pulse). After incubation, the radioactive media were removed and the cells were washed three times with PBS. The cells were then incubated with HBSS containing non-radioactive Met for 10, 25, 40 and 55 min (chase). After chase, the cell pellets were separated into water-soluble, lipid-soluble, RNA hydrolysate, DNA hydrolysate (no pathway to DNA from Met, this might be derived from basic proteins such as chromosomal histone) and protein phases. In addition, an inhibitor of protein synthesis cycloheximide was also used to pre-treat WCH17 cells for 2 hr and then repeat the pulse and chase study with [L-methyl-^{3}H]-Met while keeping cycloheximide in the media. The inhibitor cycloheximide was used to divert the pathway to PtdEtn methylation pathway instead of protein synthesis.

Results

Met can incorporate into PtdEtn methylation pathway or protein synthesis. We pulsed the WCH17 cells with L-[methyl-^{3}H]-Met followed by incubation with non-radioactive Met medium for different chase durations (Supplemental Figure 2(A)). It was found that the major intracellular H-3 activity was located in protein fraction, which is fairly stabilized within 60 min

chase duration with slightly increase. Most importantly, the labeled protein fraction was rapidly increased during the first 5 min pulse time. On the other hand, in the inhibition study, it is interesting to find that the major H-3 activity was located in water-soluble phase 60 min chase duration (Supplemental Figure 2(B)). Another observation is that the conversion from water-soluble phase to lipid (the major component is [^{3}H]-PtdCho) occurred slowly even when the protein synthesis was blocked in WCH17 cells. It is suggested that HCCs has a higher activity in CDP-Cho pathway and a disruption in PtdEtn methylation pathway. Our data are in line with this metabolic context.



Supplemental Figure 3. Effect of oleic acid and phospholipids on the PtdCho synthesis *in vitro*. (A) Incorporation of [methyl-^{14}C]-Cho into different phases in WCH17 cells. (B) Incorporation of [methyl-^{14}C]-Cho into different phases in WCH17 cells with the presence of 1mM oleic acid. (C) The effect of different combinations of oleic acid and phospholipids on the PtdCho synthesis. * vs. Control: $p < 0.05$. DOPtdCho: dioleoyl phosphatidylcholine; DOPtdEtn:

dioleoyl phosphatidylethanolamine; lysoPtdCho: lysophosphatidylcholine; lysoPtdEtn: lysophosphatidylethanolamine; PtdCho: phosphatidylcholine.

SUPPLEMENTAL FIGURE 3

It was reported that endogenous lipid contents in HCCs are different and show an increase in short/medium chain unsaturated fatty acid as compared to the surrounding hepatic tissues (*1*). The alternation of this microenvironment may affect Cho tracer metabolism *in vivo*. In order to mimic this change, we added different combinations of phospholipids and/or oleic acid into culture media of WCH17 cells to investigate PtdCho synthesis in HCC.

Materials and Methods

The 1 mM oleic acid was prepared as described by Van Harken et al. (*2*)**.** 1×10^7 WCH17 cells in 75 cm^2 corning cell culture flasks were incubated in triplicates for 5 min at 37°C with 370 KBq [methyl-^{14}C]-Cho (pulse) in Dulbecco's PBS containing $CaCl_2$ and $MgCl_2$. After incubation, the radioactive media were removed and the cells were washed three times with PBS. And the cells were then incubated with Dulbecco's PBS containing $CaCl_2$ and $MgCl_2$, 18 μM non-radioactive Cho for 10, 25, 40 and 55 min (chase). The Dulbecco's PBS contains either with or without 1 mM oleic acid. After chase, the cell pellets were separated into water soluble, lipid soluble, RNA hydrolysate, DNA hydrolysate and protein phases as described above. Metabolites analysis of intracellular fate of [methyl-^{14}C]-Cho using HPLC as described above.

The effect of phospholipids on PtdCho synthesis was also tested based on the procedure as reported previously (*3*). WCH17 cells were pre-incubated with different combinations of phospholipids (PtdCho, lysoPtdCho, DOPtdCho, DOPtdE, lysoPE) and/or oleic acid in PBS as

culture media for 30 min and then incubated with [^{14}C]-Cho in the same culture media for another 30 min. The production of [^{14}C]-PtdCho intracellularly was quantified by HPLC.

Results

The effect of oleic acid alone on the PtdCho synthesis through activating CCT was obvious. After the pulse of [^{14}C]-Cho for 5 min, the radioactivity media were removed and PBS containing cold Cho was added into WCH17 cells (chase) for different chase durations up to 55 min. HPLC analysis of PtdCho synthesis during the chase duration showed the oleic acid can significantly increase the PtdCho synthesis during the 55 min chase time. In contrast, without oleic acid, PC converted slowly to PtdCho within 55 min chase duration (Supplemental Figure 3(A)). The ratio of PC versus PtdCho decreased from 20 to 0.25 with oleic acid stimulation within 55 min chase duration (Supplemental Figure 3(B)), consistent with the function of CCT, the regulatory enzyme under this condition.

Furthermore, the effect of different combinations of phospholipids and/or oleic acid on PtdCho synthesis in WCH17 cells is showed in Supplemental Figure 3 (C). Most phospholipids combination can increase [^{14}C]-PtdCho synthesis. However, there is no significantly difference between control and PC or DOPtdCho including in the culture media.

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